

## HISTOCHEMICAL STUDIES ON MAJOR SALIVARY GLANDS IN PRENATAL BUFFALO (*Bubalus bubalis*)

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**Abstract:** The present work was conducted on the major salivary glands of 49 buffalo embryos and fetuses ranging from 40 days to 253 days. The developing ducts and secretory acini were strongly positive for PAS and AB in mandibular gland, while in parotid gland negative reaction in the acinar cells and positive reaction for PAS was noticed only in the basement membrane around the acini and ducts. The intralobular and interlobular ducts of all major salivary glands showed a negative PAS. An intense positive reaction was noticed in the terminal buds, terminal tubules and primary ducts of mandibular, parotid and sublingual glands for Alcian blue (AB). In parotid and mandibular salivary glands a positive reaction was observed for Acid and Alkaline Phosphatases in myoepithelial cells around the acini, developing ducts and basement membrane of secretory acini, while mild activity for ACP in basement membrane of acinar cells and myoepithelial cells and negative activity for ALP was noticed in sublingual salivary gland.

**Keywords:** Histochemistry, Prenatal buffalo, Major Salivary glands.

**Introduction:** Major salivary glands of buffalo are paired structures and these include parotid, mandibular and sublingual glands. The secretions of these glands are conveyed to oral cavity through different excretory ducts (Dellmann and Brown, 1998). Salivary glands contains different mucopolysaccharides, various enzymes & glycoproteins which are helps in the digestion of the food. The present study was undertaken with the aim to observe the distribution of Acid phosphatase, Alkaline phosphatase, Glycogen & sulphomucins in the salivary glands of the buffalo during prenatal development.

### MATERIALS & METHODS

Total 49 buffalo embryos and foetuses ranging from 40 to 253 days (2.5 to 79.5 cm CVRL) were used in the present study. The age of foetuses was determined on the basis of their

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CVRL by using Soliman's formula. For this histochemical study the fresh tissue pieces were collected and fixed in chilled 10% Buffered formalin and chilled acetone. Frozen sections of 15-20  $\mu$  thickness were cut with cryostat and subjected to the following histochemical staining methods Oil-red 'O' in Propylene glycol for lipids (Luna, 1968), Gomori's cobalt method for alkaline phosphatase (Pearse, 1968), Gomori's cobalt method for acid phosphatase (Pearse, 1968) and Alcian blue (PH<sup>1</sup>) method for sulphomucins (Culling, 1974).

## RESULTS & DISCUSSION

### Parotid salivary gland

Histochemically, Periodic acid schiff's (PAS) reagent did not reveal any reaction in the acinar cells of parotid salivary gland, while basement membrane around the acini and ducts showed positive reaction. A positive reaction for Alcian blue (AB) was noticed in the terminal buds, terminal tubules and primary ducts at 110 days (Fig.1) but Suzuki *et al.*, (1983) found positive and negative activities for PAS and AB respectively in the acinar cells of bovine parotid gland, while Shackelford and Klapper (1962) noticed lack of acid mucopolysaccharides in the cow. Mild positive reaction was observed for AB in early embryonic mesenchymal tissue around the terminal buds. A weak reaction was noted in the interlobular connective tissue and a positive reaction in the secretory acini and ducts of late age groups. However Boshell and Willborn (1978) reported an absence of reaction with sulphomucins in the parotid gland of pig.

The existence of myoepithelial cells around the acini and intercalated ducts was revealed by their positive reaction for ALP and ACP. The presence of only ALP activity was reported to be noticed in the myoepithelial cells around the acini and ducts of parotid glands of sheep and lambs by Silver (1954). However in the present study the glandular cells showed a negative reaction for ACP and ALP. A strong positive reaction was noticed in ducts and basement membrane of the glandular cells for ALP and ACP and mild positive reaction for ALP was observed in the blood vessels, secretory contents of the ducts and interlobular connective tissue.

### Mandibular Gland

Histochemically, the epithelial portions of the primary ducts and terminal buds of mandibular salivary gland were negative for PAS in early age groups while PAS Positive in PAS positive as in human beings El- Mohandes *et al.*, (1987) and buffalo Venkatakrishnan (1994). The parenchyma showed intense positive reaction in the acini and ducts for PAS staining at 158 days (Fig. 02) as reported in buffalo Venkatakrishnan (1994). The developing ducts and acini

were strongly positive for AB which agree with the reports of El-Mohandes (1987) in human beings. The large intralobular and interlobular ducts were negative for PAS. Positive intense reaction was observed in the terminal tubules, acini and ducts for Alcian blue (PH<sup>1</sup>) at 93 days (Fig. 03) these findings were correlated with the findings of the Shackelford & Bentley (1963).

The glandular cells showed no reaction for ACP and ALP. An intense positive reaction for ALP was noticed in the ducts of the gland, interlobular connective tissue, ducts and myoepithelial cells around the acini and intercalated ducts at 208 days this findings will correlated with the findings of Shear (1964) in rat submandibular gland. Mild positive reaction was noticed for ALP in the connective tissue between the lobules and around the acini and ducts which was not reported earlier Positive intense reaction was noticed in the ducts while mild positive reaction was observed in the basement membrane and myoepithelial cells for ACP at 220 days (Fig. 04).

### **Sublingual Gland**

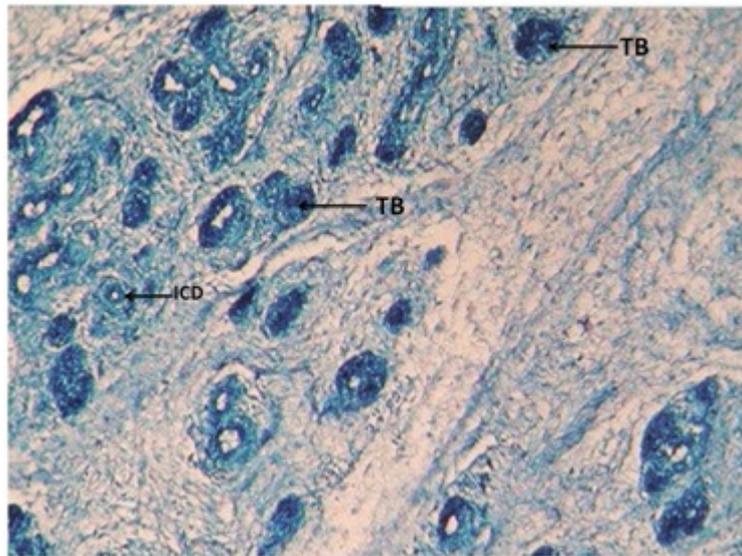
No activity of the PAS activity were observed in the early and mid foetal age groups of sublingual salivary gland but the a mild positive staining for PAS was observed in the apical portions of the cells of the acini and ducts at 188 days (Fig. 05), Intense positive reaction for sulphomucins was noticed only in the terminal tubules and primary ducts for AB as reported in buffalo by Pal *et al.* (1972).

The basement membrane of acinar cells and myoepithelial cells showed a mild positive ACP activity at 220 days (Fig.06) but no reaction was observed for ALP.

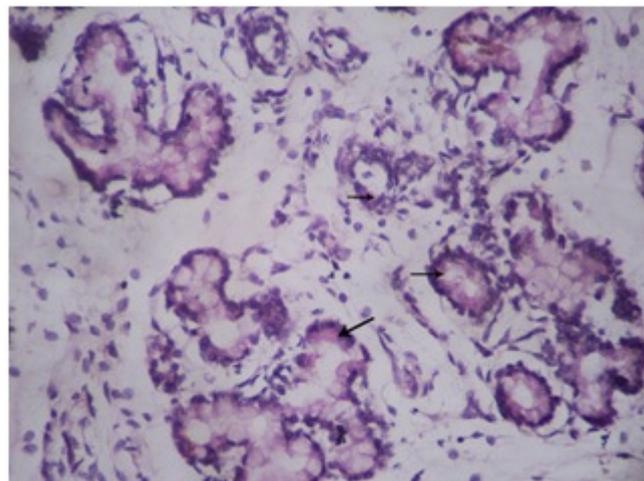
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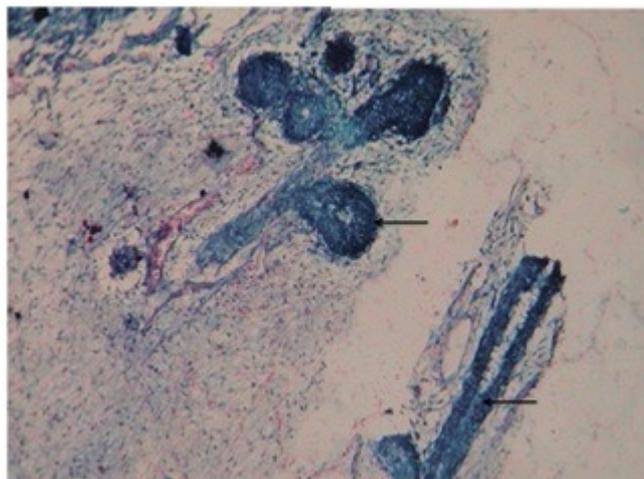
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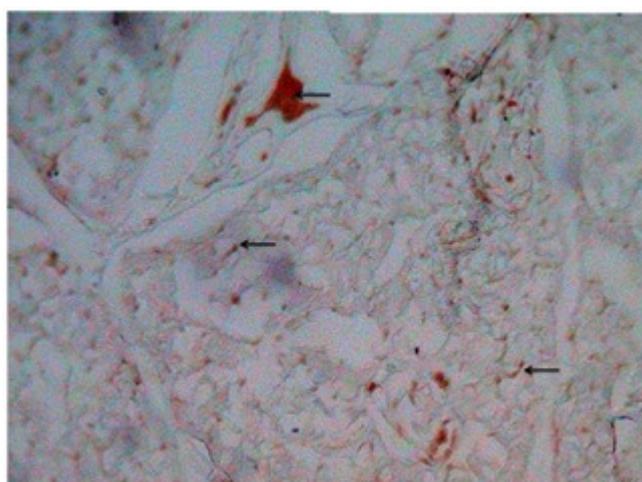
**Fig.1** Cross section of foetal parotid gland showing positive reaction for sulphomucins in terminal bud (TB) and intercalated ducts (ICD) at 110 days. AB X100.



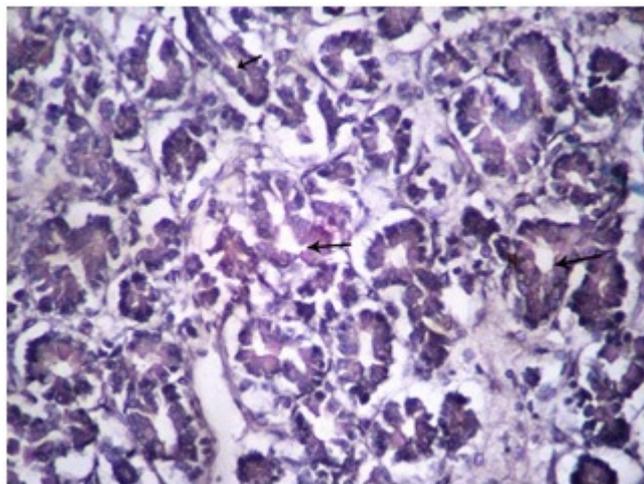
**Fig.2** Photomicrograph of 158 day foetal mandibular gland showing positive intense reaction in the acini and ducts (Arrow) for PAS. PAS X 200.



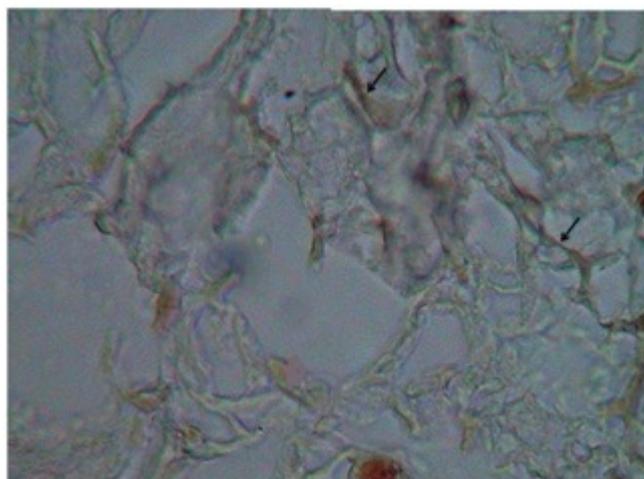
**Fig.03.** Photomicrograph of foetal mandibular gland showing positive intense reaction for AB (Arrow) in the terminal tubules and ducts at 93 days. AB X100



**Fig.04** Photomicrograph of 220 day foetal mandibular gland showing positive intense reaction for ACP (Arrows) in the ducts and mild reaction in the myoepithelial cells and basement membrane at 220 days. Gomoris cobalt method for acid phosphotase X100



**Fig 05** Photomicrograph of foetal sublingual gland showing mild positive reaction for PAS (Arrow) in acinar cells and ducts at 188 days. PAS X100.



**Fig.06** Photomicrograph of foetal sublingual gland showing mild positive activity for ACP (Arrow) in the myoepithelial cells and basement membrane at 220 days. Gomoris cobalt methods for acid phosphotase X100.